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Minireview

Endogenous cannabinoids: Metabolism and their role in reproduction

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Abstract

Over the past two decades a number of endogenous compounds that act as ligands for the cannabinoid receptors has been discovered. In analogy with the "endorphins" these compounds have been called "endocannabinoids". Endocannabinoids have been demonstrated in many mammalian tissues including humans and are widely distributed in the CNS, peripheral nerves, uterus, leukocytes, spleen and testicles. The uterus contains the highest levels of anandamide, the first discovered endocannabinoid, suggesting an important role for this substance in reproduction. Several studies have shown anandamide to be involved in the regulation of implantation and reduced activity of the enzyme that degrades anandamide has been associated with early pregnancy loss in humans. The bulk of the literature concerning endocannabinoids is based upon anandamide related studies; therefore, in this review we focus on the metabolism of anandamide and its role in reproduction.

Keywords: Anandamide; Fatty acid amide hydrolase; Lymphocytes/Uterus

Introduction

Endogenous cannabinoids are unsaturated fatty acid derivatives which act as endogenous ligands for cannabinoid receptors. The first product of this group, Anandamide (arachidonylethanolamide), was isolated from the porcine brain in 1992 [1]. The name was coined

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from the Sanskrit word "Ananda" meaning bliss. Since then a number of fatty acid derivatives that belong to the group, endocannabinoids has been isolated. These include sn-2 arachidonyl-glycerol [2,3,4], homo-γ-linoleoylethanolamide and docosatetraenoyl-ethanolamide [5].

Anandamide has several recognised effects on the nervous system. Through interactions and modulation at the level of neurotransmitters in different regions of the nervous system [6] it produces several effects on various brain activities. In rodents for example, anandamide inhibits memory consolidation [7], impairs working memory [8], exhibits analgesic properties [2], inhibits motor coordination (See Ref. [6] for review) and anxiety like responses [9] and decreases arousal [10]. All these effects suggest that it might act as a "stress recovery-factor" relieving some of the stress- induced responses and sending messages such as "relax, eat, sleep, forget and protect" (Cited from [6]).

The uterus contains the highest concentrations of anandamide yet discovered in mammalian tissues [11] and this suggests that it might play a role in reproduction. Although there is a dearth of knowledge on the role of endocannabinoids in reproduction, the fact that exogenous cannabinoids such as marijuana and hashish have significant effects on reproduction and pregnancy suggests a potential role for endocannabinoids in reproduction. In human marijuana smoking during pregnancy is significantly associated with an increased risk of preterm labour [12] and intrauterine growth restriction (evidenced by reduction of birth weight, height, head circumference, mean arm muscle circumference and non-fat area of the arm) [13,14,15]. Acute marijuana smoking in humans suppresses plasma LH in the luteal phase. In chronic users, it shortens the menstrual cycle, the effect being predominately a short luteal phase leading to menstrual irregularities and anovulation [16].

Metabolism of anandamide

Analytical studies and tissue distribution

In the human, anandamide has been isolated in the brain, spleen, heart [17] and breast cancer cells [18]. Its distribution is more widespread in the rat where it has been found in phaeochromocytoma cells [18], the kidneys [19], brain [17,20], testicles [21], spleen [17], skin [17] and blood plasma [22]. It has also been found in the murine uterus [11], leukocytes and bovine and porcine brain [23]. The highest concentrations of anandamide in the rat brain are found in the hippocampus and striatum [17].

Synthesis of anandamide

Anandamide is an ethanolamine amide of arachidonic acid, the precursor of prostaglandins and leukotrienes. It is produced on demand and released from cells when the need arises (i.e. not stored in cells) [24]. The exact stimulus for its synthesis is poorly understood. Two mechanisms for the production of anandamide have been described. The first involves the hydrolysis of a phospholipid precursor in the cell membrane called N-arachidonyl-phospha-

tidyl ethanolamine (NAPE) to anandamide (Fig. 1). This process is mediated by the enzyme N-acyl-phosphatidyl ethanolamine selective phospholipase D (Na PE-PLD) [6,21,25–27]. This mechanism is initiated by calcium ion influx into the cell and has been shown to occur in several mammalian tissues including cultured intact rat neurons [25] and in rat testes [21].

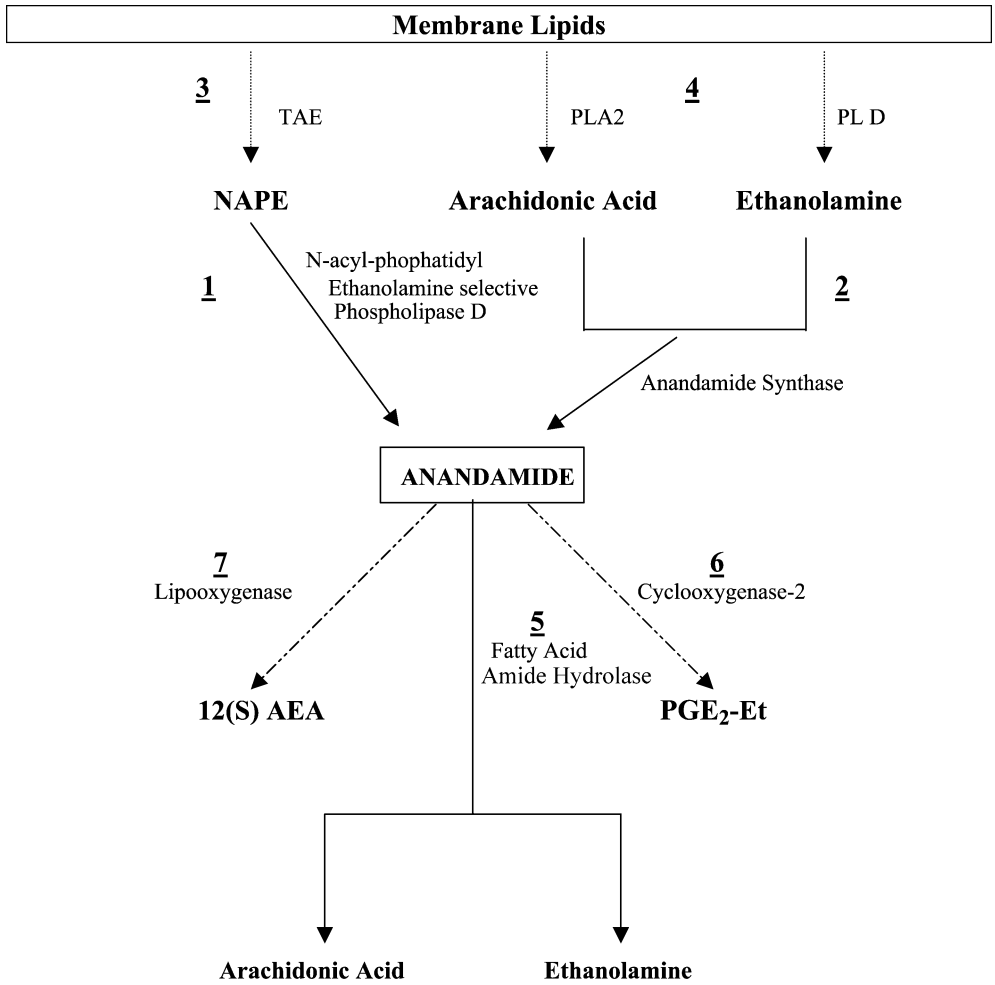

Fig. 1. **Metabolism of Anandamide. 1)** Anandamide production by Na-PE-PLD from N-arachidonyl-phosphatidyl ethanolamine. **2)** Anandamide production by Anandamide Synthase. **3)** N-arachidonyl-phosphatidyl ethanolamine is produced by the activity of transacylase enzyme. **4)** Production of arachidonic acid and ethanolamine from membrane lipid precursors. **5)** Degradation of anandamide by fatty acid amide hydrolase. **6)** Degradation of anandamide by cyclooxygenase-2 enzyme. **7)** Degradation of anandamide by lipooxygenase enzyme. Abbreviations: TAE, (Transacylase enzyme); PLA-2, (Phospholipase-2 enzyme); PLD, (Phospholipase D enzyme); NAPE, (N-arachidonyl-phosphatidyl ethanolamine); PGE_2-Et, (Prostaglandin E2-ethanolamine); 12(S) AEA, (12(S)-hydroxy-arachidonylathanolamide).

The precursor NAPE is produced in the cell membrane by a Ca^{2+}-dependent transacylase enzyme [6,26,27]. The second mechanism which is de-novo synthesis of anandamide from arachidonic acid and ethanolamine by condensation (Fig. 1) has however not been demonstrated in living cells [6].

Release and uptake of anandamide

Once synthesized, anandamide is released from the cell into the extra cellular space where it can either act in an autocrine or paracrine manner [24]. Due to its lipophylic nature, anandamide may diffuse passively through the cell membrane into the cystol [28]. It can also be internalised by the process of facilitated diffusion mediated by a selective anandamide transporter - this indeed is the main mechanism for internalisation of anadamide [29,30]. Several compounds, for example phenylmethylsulfonyl fluoride, oleamide [30,31] and *N*-(4-hydroxyphenyl) arachidonoylamine (AM404) [29]) can inhibit anadamide uptake and therefore potentiate its effects.

Degradation of anandamide

Once internalised anandamide is metabolised to arachidonic acid and ethanolamine (Fig. 1) by fatty acid amide hydrolase (FAAH) [32,33]. This enzyme, originally known as ''anandamide hydrolase'' and ''anandamide amidohydrolase'', is encoded by a single gene localised on the short arm of human chromosome one [34]. It is 579 amino acids long and has a molecular mass of 63.0 kDa [33]. It is most abundant in brain and liver tissues and is present to a lesser extent in the spleen, kidney, testis and lungs [28,34].

Human FAAH shares 82% and 84% sequence identity with FAAH in the rat and mouse respectively. This degree of sequence homology suggests that the enzymatic mechanism for anandamide metabolism has been well conserved through mammalian evolution [28,34]. FAAH is inhibited by phenylmethylsulfonyl fluoride (PMSF), arachidonic acid, arachidonyl trifluromethyl ketone, 2-octyl γ-bromoacetoacetate, and palmitylsulfonylflouride (AM374) [35–40].

Anandamide is also metabolised by cyclo-oxygenase - 2 to prostaglandin E_2-ethanolamide [41]. Although the physiological significance of this metabolic pathway is unclear, it does, however, further suggest a close link between anandamide and eicosanoids. Anandamide is also metabolised by lipo-oxygenase to form 12 (S)-hydroxy-arachidonylethanolamide, but the physiological significance of this is unclear [42–44].

Mechanism of action

Cannabinoid receptor-mediated actions

Anandamide acts as an agonist for cannabinoid receptors. Two subtypes of receptors have been described to date. The first, CB_1, was first described and characterised in the rat brain

[45]. The cloning and expression of a complementary DNA from a rat brain cDNA library that encoded CB_1 was reported in 1990 [46]. The human CB_1 shares 97.3% sequence identity with the rat CB_1 with 100% identity within the trans-membrane regions [47]. The second receptor subtype, CB_2, was first characterised from macrophages of the marginal zone of the spleen [48]. The CB_2 receptor shows 48% identity with CB_1 with 68% identity within the trans-membrane regions.

CB_1, originally called central receptor, is the main receptor in the brain. It is found in several areas of the brain including the hippocampus (which probably explains the effects of cannabinoids on memory functions), cerebellum, basal ganglia and cerebral cortex. Other tissues that express CB_1 include the cervical ganglion, peripheral autonomic nerves, testes, spleen, peripheral leukocytes, sperm, uterus, vascular endothelial and smooth muscle cells, human eye (retina, iris, corneal epithelium and cilliary body) and human placenta [47,49–58]. The CB_2 receptor, on the other hand, was first isolated from splenic cells; hence it is called "spleen-type" cannabinoid receptor. It is found in a number of immune system-derived cells including some T-cell lines (EL4.IL2, Jurkat E6-1 and HPB-ALL) [59], monocyte lines HL60 [48] and RAW264.7 [60] and the mast cell line RBL-2H3 [61]. Both receptors belong to the super family of G-protein coupled receptors (GPCR) and their functional responses are mediated by pertussis toxin sensitive GTP-binding ($G_{i/o}$) proteins. Two effects of stimulation of the CB receptors are recognized. Firstly, inhibition of adenylate cyclase leading to decreased levels of c-AMP [62–66], and secondly, inhibition of N-type and P/Q type calcium channels leading to a reduction of calcium ion influx to the cells [63,65–68]. These two receptor sub-types can be selectively inhibited/antagonised. SR141716A for example, given orally antagonises the CB_1receptor while SR 144528 antagonises the CB_2 receptor [24,28]. Certain effects of anandamide, for example, activation of arachidonic acid release and inhibition of gap-junction mediated Ca^{2+} signalling in astrocytes are not mediated by either of the two known receptors but are mediated by interactions with G-proteins [69].

Non-cannabinoid receptor mediated actions

Anandamide can also affect cellular functions without interacting with cannabinoid receptors [28]. It can liberate arachidonic acid and positively modulate N-methyl-D-aspartate (NMDA)-mediated Ca^{2+} currents in cells that do not express the cannabinoid receptors [70]. In rabbit skeletal muscles, rat cardiac and brain membranes, anandamide inhibits L-type Ca^{2+}-channels by direct interaction with dihydropyridine binding sites of these channels [71,72].

Effects of endogenous cannabinoids on reproduction

Effects on the hypothalamic-pituitary-ovarian axis

Exocannabinoids have several adverse effects on the hypothalamic-pituitary-ovarian axis in both animals and humans. As mentioned earlier, marijuana smoking is associated with the

suppression of LH and a shortened luteal phase in humans [16,73]. Delta-9-Tetrahydrocannabinol (Δ^9-THC) causes delayed puberty in the female rat [74]. Exposure to Δ^9-THC suppresses the preovulatory surge of LH in the female rat leading to blocked ovulation [75]. Chronic exposure to Δ^9-THC in the female rat causes irregular oestrous cycles with reduced serum LH and FSH [76,77] and affects the ultra structure of preovulatory follicles, with a reduction in the mitochondrial and lipid droplet volumes in peripheral granulosa cells [78]. Similar findings of suppression of serum LH and delayed ovulation have been described in the female Rhesus monkey [79]. The inhibitory effect on ovulation is reversed by the administration of gonadotrophin releasing hormones [76]. This suggests that the inhibitory effect is mediated via the inhibition of hypothalamic factors, which naturally stimulate the release of pituitary LH.

The effects of endogenous cannabinoids on the hypothalamic-pituitary-ovarian axis are quite similar to those of Δ^9-THC. Anandamide decreases serum LH and prolactin levels in rats of both sexes, although it does not affect the level of serum FSH [80]. Initially, the site of action of anandamide was thought to be the hypothalamic regulatory centres with no direct effect on the anterior pituitary. More recently, the CB_1 receptor was identified in the anterior pituitary itself [81,82] and suggests that anandamide may affect the function of the anterior pituitary by direct action as well as by interfering with the hypothalamic regulatory factors; the latter mechanism is supported by the fact that cannabinoid receptors are present in the arcuate nucleus and the preoptic area [83]. The expression of the CB_1 receptor gene in the anterior pituitary is regulated by sex steroids [84]. The amounts of CB_1 receptor-mRNA transcripts in the anterior pituitary fluctuate through the ovarian cycle in rats with the highest levels in the diestrus and the lowest in the oestrus phase. In contrast, anandamide levels in the anterior pituitary peak in the estrus phase and are lowest in the diestrus phase. The concentration of anandamide in the hypothalamus shows the reversed pattern, suggesting that anandamide concentration in both the anterior pituitary and the hypothalamus are influenced by sex steroids [84]. Anandamide might, therefore, play an important role in the regulation of the hypothalamic-pituitary-ovarian axis, possibly affecting the process of ovulation and the regulation of the menstrual cycle.

Anandamide affects the neuroendocrine system of pregnant rats. When administered chronically it prolongs the duration of pregnancy and increases the rate of stillbirth [85]. It also reduces the levels of serum LH, pituitary prolactin, serum prolactin, serum progesterone and prostaglandins PGF_1 and PGF_2 [85]. The reduction in the levels of prostaglandins might partly explain the prolongation of pregnancy. The prolongation of the duration of the pregnancy is different from the effect of exogenous cannabinoids which, as mentioned earlier, are associated with preterm labour. Administration of anandamide has no effect on the levels of FSH or gonadotrophin releasing hormone [85]. The postnatal development of the hypothalamic pituitary axis in the offspring of animals who receive anandamide during the pregnancy is temporarily inhibited [86]. The inhibitory effect is more prominent in males compared to females [86]. This inhibitory effect on the developing hypothalamic pituitary axis disappears by the 20th postnatal day [86].

Effects of endogenous cannabinoids on spermatogenesis, fertilisation, embryo development and pregnancy implantation

In animal studies, both anandamide and Δ^9-THC have been demonstrated to have adverse effects on sperm function. They reduce sperm fertilizing capacity of sea urchins by inhibiting the acrosome reaction, an effect mediated by the CB_1 receptor [51,52]. Chronic exposure to exogenous cannabinoids in mice leads to impaired spermatogenesis at both the mitotic and meiotic stages [87]. Chronic marijuana use in humans is associated with reduced serum testosterone levels, reduced sperm counts and impotence [88]. Marijuana use in early pregnancy in humans is associated with increased incidence of fetal loss [16]. Several animal studies have demonstrated adverse effects of marijuana exposure in early pregnancy. Marijuana exposure in the rabbit for example, is associated with an increased rate of embryo resorption [89]. The murine uterus has the capacity to synthesise anandamide (*de novo* synthesis) and indeed contains the highest level of anandamide yet discovered in mammalian tissues [11]. The CB_1 receptor-mRNA is expressed in the epithelial cells of the mouse uterus [53]. The uterus becomes receptive for implantation on day 4 (implantation day) and by day five it becomes non-receptive [90]. The concentrations of anandamide and the enzymatic activities involved in the synthesis and degradation of anandamide show little changes in the first four days of pregnancy (before implantation) apart from a significant decrease in FAAH activity between days three and four (Fig. 2a, b and c).

After implantation on day four, anandamide concentrations in the implantation and non-implantation sites diverge with non-implantation site levels increasing dramatically with a concomitant increase in anandamide synthase activity and a decrease in FAAH activity (Fig. 2a, b and c). As a result of these changes, the concentrations of anandamide and the anandamide synthase activity are significantly higher in the non-implantation sites compared to the implantation sites (Fig. 2a and b). FAAH activity is also higher in the implantation sites compared to the non-implantation sites (Fig. 2c). Analysis of anandamide concentrations and the enzymatic activities in pseudopregnant uteri on day five reveals findings similar to those encountered at the non-implantation sites [11,91]. In keeping with these findings, FAAH expression has been found to be reduced on day five compared to the first four days of pregnancy [92]. These findings suggest that the systemic endocrine milieu in early pregnancy is involved in the increased levels of anandamide and the enzymatic changes described. Indeed, these findings are supported by the recent observation that oestrogen and progesterone derived from the maternal ovary suppress the activity of FAAH in the mouse uterus [93]. The fact that the implantation and non-implantation sites, and the pseudopregnant uterus have different concentrations of anandamide and also show different enzymatic profiles suggests that local factors such as uterine decidualisation or the presence of the embryo prevents the increase of anandamide concentrations.

Both CB_1 and CB_2 mRNAs are expressed in the preimplantation mouse embryo [94]. Anandamide arrests the development of embryos at or after the 2-cell stage in-vitro, primarily between the 4-cell and 8-cell stages. The effect is dose dependent [94]. A reduction in trophoectodermal cell numbers is seen in those embryos that escape the developmental arrest in the presence of anandamide and develop into blastocysts [95]. Anandamide also reduces

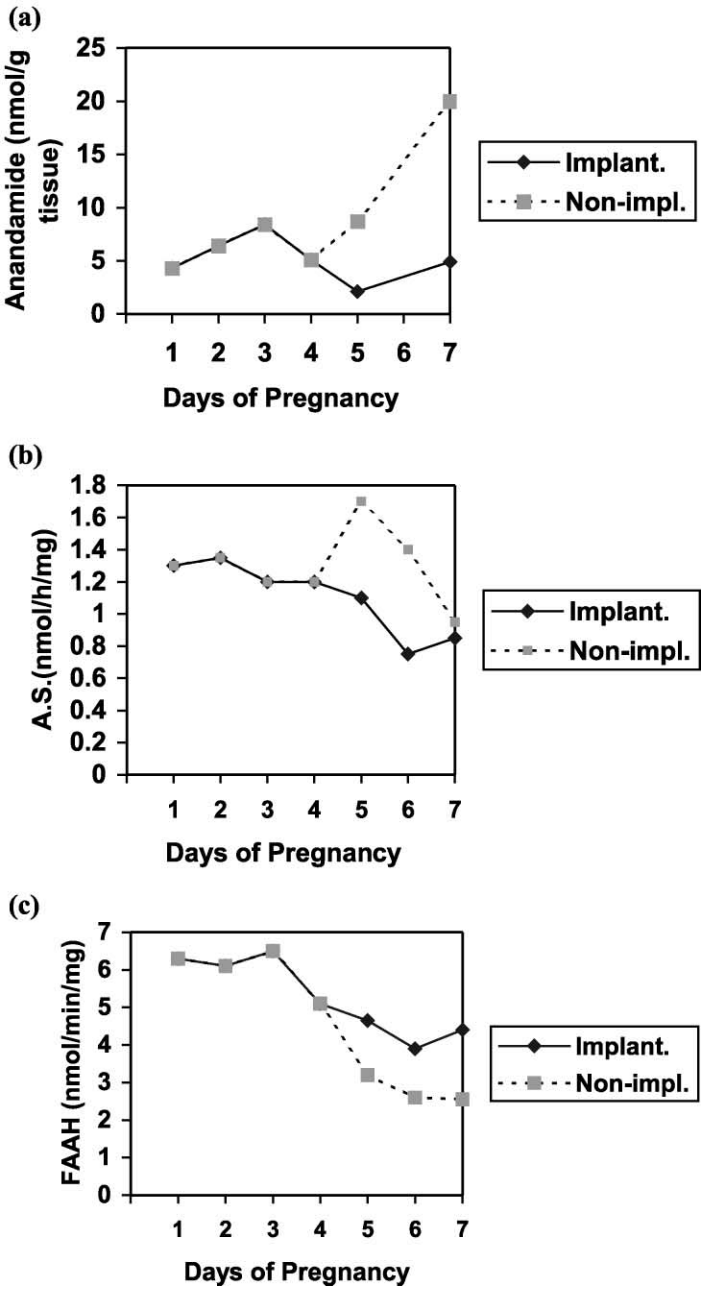

Fig. 2. Changes in Anandamide levels (a), Anandamide synthase (b) and FAAH (c) in Mouse pregnancy. Abbreviations: non-impl, non-implantation sites; implant, Implantation sites; FAAH, Fatty Acid Amide Hydrolase enzyme; A.S., anandamide synthase enzyme. Anandamide concentrations are in nmol/g tissue. (Data in 2 (a) derived from reference [11] with permission from National Academy of Sciences, U. S. A. ‘‘Copyright 1997’’. Data in 2 (b) 2 (c) derived from reference [91] with permission of Wiley-Liss, a subsidiary of John Wiley and sons, ‘‘Copyright 1996’’.

the rate of zona- hatching of blastocysts in-vitro [11]. These effects are mediated by the CB_1 receptor as they are abolished when SR141716A (CB_1 antagonist) is co-administered with anandamide [95], but not when SR144528 (CB_2 antagonist) is co-administered with anandamide [96]. The exact role of the CB_2 receptor in the embryo is yet to be defined [97]. Embryos at the 1 and 2-cell stages and at the blastocyst (day 4) stage all express FAAH, but the enzyme is not expressed in embryos at the 8-cell stage [92]. This finding suggests that FAAH in the embryo plays an important role in controlling the levels of anandamide to curtail the effects of anandamide on the embryo's development. Embryos at the blastocyst stage exposed in culture to low levels of anandamide show accelerated trophoblast differentiation and outgrowth while higher concentrations of anandamide inhibits trophoblast differentiation [98]. There are no data on the levels of anandamide and the enzymatic activities beyond day seven of the mouse pregnancy.

Anandamide maybe involved in the regulation of the "window" of implantation by synchronising embryo development with preparation of the uterus up to the receptive state [11]. The higher levels of anandamide in the non-implantation sites could be responsible for the inhibition of trophoblast proliferation while the lower levels at the implantation sites might help the proliferation of trophoblast, hence two different effects of anandamide are observed depending on its local concentrations [97]. On the other hand, excessive anandamide levels might induce the non-receptive phase prematurely leading to early pregnancy failure. In embryos that are deficient in CB_1 and/or CB_2 receptors, development is asynchronous with the uterine development [99]. On the morning of day four, 98% of wild embryos are at the blastocyst stage compared to only 62%, 71% and 61% of embryos that are deficient in CB_1, CB_2 or both CB_1 and CB_2 respectively [99]. This further supports the theory that anandamide may regulate the window of implantation. Another possible role for anandamide is that it might act acts as a reservoir of arachidonic acid for the generation of prostaglandins which are important for the embryo development and implantation [11]. Whether anandamide plays a role in the regulation of implantation in humans remains uncertain, as the mechanism of implantation in the mouse is distinctly different from that in humans. However, a recent study on the levels and activity of FAAH in maternal lymphocytes suggests that FAAH in the lymphocytes might be a useful marker for early pregnancy failure. Levels of FAAH were measured in 50 women in early pregnancy (7–11 weeks gestation) and were found to be significantly lower in 7 women who miscarried compared to that in the 43 women who had successful pregnancies [100]. From the results of this study, a threshold value for FAAH activity was identified. In a later study by the same group, the enzyme activity was measured in 150 healthy pregnant women at 8 weeks gestation. In 15 of these women the activity of the enzyme was below the threshold and all of them miscarried. In contrast, only one woman out of 135 who had FAAH activity above the threshold miscarried [100]. These findings are interesting particularly as they imply that a reduced activity of FAAH is associated with a poor outcome in contrast to the findings in the mouse that FAAH activity is suppressed in the uterus with the advancement of the pregnancy. More recently, in a study by the same group, it was found that progesterone but not β-HCG stimulates the activity of FAAH [101]. In the same study, it was shown that anandamide inhibits the production of Leukaemia-inhibitory factor (LIF) [101]. Since LIF is reported

to be defective in cases of recurrent miscarriages [102], a low level of FAAH might lead to spontaneous miscarriage by increasing the levels of anandamide which in turn inhibits the production of LIF [101]. What remains unclear at this stage is whether the changes in FAAH in early pregnancy failure are a result of the failing pregnancy or a cause of the failure. It is well recognized that failed pregnancies are associated with decreased levels of progesterone and it is possible that the reduced levels of progesterone leads to a reduction of FAAH in peripheral lymphocytes [103]. There are no studies, however, that evaluate the association between anandamide levels in the peripheral lymphocytes, the uterus and placenta.

Future Directions

Endocannabinoids are potentially an exciting group of endogenous mediators that are involved in the regulation of mammalian reproduction. The involvement of anandamide in the regulation of the anterior pituitary hormones, the close link between sex steroids and anandamide levels in both the anterior pituitary and the hypothalamus, and the fluctuation of anandamide levels throughout the ovarian cycle all suggest an important role for anandamide in the control of the menstrual cycle. If similar findings are confirmed in humans, then this might lead to a better understanding of menstrual disorders and certain cases of anovulation and infertility.

Anandamide plays a critical role in the regulation of early pregnancy acceptance and maintenance in the animal model. The exact role in humans is yet to be elucidated but the recent findings of a correlation between FAAH activity in peripheral lymphocytes and the outcome of pregnancy suggest that endocannabinoids might play an important role in the regulation/maintenance of early pregnancy. There is need for further work to explore the relation between the levels of anandamide and FAAH in lymphocytes from the uterus. Anandamide levels are influenced by sex steroid hormones. Levels of anandamide may, therefore, differ in and outside pregnancy. If this is the case, then it might explain some of the mood changes encountered during pregnancy and after childbirth. There is a need to investigate all these unanswered questions on the role of anandamide in reproduction.

References

1. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258(5090):1946–9.
2. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochemical Pharmacology 1995;50(1):83–90.
3. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochemical and Biophysical Research Communications 1995;215(1):88–97.

4. Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. Nature 1997;388(6644):773–8.
5. Hanus L, Gopher A, Almog S, Mechoulam R. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. Journal of Medicinal Chemistry 1993;36(20):3032–4.
6. Di Marzo V, Meleck D, Bisogno T, De Petrocellis L. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory actions. Trends in Neurosciences 1998;21(12):521–8.
7. Castellano C, Cabib S, Palmisano A, Di Marzo V, Puglisi-Allegra S. The effects of anandamide on memory consolidation in mice involve both D_1 and D_2 dopamine receptors. Behavioural Pharmacology 1997;8(8):707–12.
8. Mallet PE, Beninger RJ. The endogenous cannabinoid receptor agonist anandamide impairs memory in rats. Behavioural Pharmacology 1996;7:276–84.
9. Navarro M, Hernandez E, Munoz RM, del Acro L, Villanua MA, Carrera MR, Rodriguez de Fonseca F. Acute administration of the CB_1 cannabinoid receptor antagonist SR141716A induces anxiety-like responses in the rat. NeuroReport 1997;8(2):491–6.
10. Santucci V, Strome JJ, Soubrie P, Le Fur G. Arousal-enhancing properties of the CB_1 receptor antagonist SR 141716A in rats as assessed by electroencephalographic spectral and sleep-waking cycle analysis. Life Sciences 1996;58(6):PL103–10.
11. Schmid PC, Paria BC, Krebsbach RJ, Schmid HH, Dey SK. Changes in anandamide levels in mouse uterus are associated with uterine receptivity for embryo implantation. Proceedings of the National Academy of Sciences of the United States of America, vol. 94(8). 1997. pp. 4188–92.
12. Gibson GT, Baghurst PA, Colley DP. Maternal alcohol, tobacco and cannabis consumption and the outcome of pregnancy. Australian and New Zealand Journal of Obstetrics and Gynaecology 1983;23(1):15–9.
13. Zukerman B, Frank DA, Hingson R, Amaro H, Levenson SM, Kayne H, Parker S, Vinci R, Aboagye K, Fried LE, Cabral H, Timperi R, Bauchner H. Effects of maternal marijuana and cocaine use on fetal growth. New England Journal of Medicine 1989;320(12):762–8.
14. Frank DA, Bauchner H, Parker S, Huber AM, Kyei-Aboagye K, Cabral H, Zukerman B. Neonatal body proportionality and body consumption after in utero exposure to cocaine and marijuana. The Journal of Pediatrics 1990;117(4):622–6.
15. Sherwood RA, Keating J, Kavvadia V, Greenough A, Peters TJ. Substance misuse in pregnancy and relationship to fetal outcome. European journal of Pediatrics 1999;58(6):488–92.
16. Mendelson JH, Mello NK, Ellingboe J, Skupny AS, Lex BW, Griffin M. Marihuana smoking suppresses luteinizing hormone in women. Journal of Pharmacology and Experimental Therapeutics 1986;237(3):862–6.
17. Felder CC, Nielsen A, Briley EM, Palkovits M, Priller J, Axelrod J, Nguyen DN, Richardson JM, Riggin GA, Koppel GA, Paul SM, Becker GW. Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. FEBS letters 1996;393(2–3):231–5.
18. Bisogno T, Katayama K, Melck D, Ueda N, De Petrocellis L, Yamamoto S, Di Marzo V. Biosynthesis and degradation of bioactive fatty acid amides in human breast cancer cells and rat pheochromocytoma cells-implications for cell proliferation and differentiation. European journal of Biochemistry 1998;254(3):634–42.
19. Deutsch DG, Goligorsky MS, Schmid PC, Krebsbach RJ, Schmid HH, Das SK, Dey SK, Arreaza G, Thorup C, Stefano G, Moore LC. Production and physiological actions of anandamide in the vasculature of the rat kidney. The journal of Clinical Investigation 1997;100(6):1538–46.
20. Koga D, Santa T, Fukushima T, Homma H, Imai K. Liquid chromatographic-atmospheric pressure chemical ionization mass spectrometric determination of anandamide and its analogs in rat brain and peripheral tissues. Journal of Chromatography. B, Biomedical Applications 1997;690(1–2):7–13.
21. Sugiura T, Kondo S, Sukagawa A, Tonegawa T, Nakane S, Yamashita A, Waku K. Enzymatic synthesis of anandamide, an endogenous cannabinoid receptor ligand, through N-acylphosphatidylethanolamine pathway in testis: involvement of Ca^{2+}- dependent transacylase and phosphodiestrase activities. Biochemical and Biophysical Research Communications 1996;218(1):113–7.
22. Giuffrida A, Piomelli D. Isotope dilution GC/MS determination of anandamide and other fatty acylethanolamines in rat blood plasma. FEBS Letters 1998;(3):373–6.

23. Schmid PC, Kerbsbach RJ, Perry SR, Dettmer TM, Maasson JL, Schmid HH. Occurrence and post mortem generation of anandamide and other long-chain N-acylethanolamines in mammalian brain. FEBS letters 1995;375(1–2):117–20.
24. Piomelli D, Giuffrida A, Calignano A, Rodriguez de Fonseca F. The endocannabinoid system as a target for therapeutic drugs. Trends in Pharmacological Sciences 2000;21(6):218–24.
25. Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 1994;372(6507):686–91.
26. Fride E, Mechoulam R. Ontogenic development of the response to anandamide and delta 9-tatrahydrocannabinol in mice. Brain Research. Developmental Brain Research 1996;95(1):131–4.
27. Di Marzo V, De Petrocellis L, Bisogno T, Melck D. Metabolism of anandamide and 2- arachidonoylglycerol: a historic overview and some recent developments. Lipids 1999;34:S319–25 (Suppl).
28. Di Marzo V. Endocannabinoids and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. Biochimica et Biophysica Acta 1998;1392(2–3):153–75.
29. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport as revealed by selective inhibition. Science 1997;277(5329):1094–7.
30. Piomeli D, Beltramo M, Glasnapp S, Lin SY, Goutopoulos A, Xie XO, Makriyannis A. Structural determinants for recognition and translocation by the anandamide transporter. Proceedings of the National Academy of Sciences of the United States of America, vol. 96(10). 1999. pp. 5802–7.
31. Jarrahian A, Manna S, Edgemond WS, Campbell WB, Hillard CJ. Structure-activity relationships among N-arachidonylethanolamine (Anandamide) head group analogues for the anandamide transporter. Journal of Neurochemistry 2000;74(6):2597–606.
32. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 1996;384(6604):83–7.
33. Giang DK, Cravatt BF. Molecular characterization of human and mouse fatty acid amide hydrolases. Proceedings of the National Academy of Sciences of the United States of America, vol. 94(6). 1997. pp. 2238–42.
34. Wan M, Cravatt BF, Ring HZ, Zhang X, Francke U. Conserved chromosomal location and genomic structure of human and mouse fatty-acid amide hydrolase genes and evaluation of clasper as a candidate neurological mutation. Genomics 1998;54(3):408–14.
35. Deutsch DG, Chin ZA. Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. Biochemical Pharmacology 1993;46(5):791–6.
36. Koutek B, Prestwich GD, Howlett AC, Chin SA, Salehani D, Akhavan N, Deutsch DG. Inhibitors of arachidonoyl ethanolamide hydrolysis. Journal of Biological Chemistry 1994;269(37):22937–40.
37. Childers SR, Sexton T, Roy MB. Effects of anandamide on cannabinoid receptors in rat brain membranes. Biochemical Pharmacology 1994;47(4):711–5.
38. Adams IB, Ryan W, Singer M, Thomas BF, Compton DR, Razdan RK, Martin BR. Evaluation of cannabinoid receptor binding and in vivo activities for cannabinoid analogs. Journal of Pharmacology and Experimental Therapeutics 1995;273(3):1172–81.
39. Patricelli MP, Patterson JE, Boger DL, Cravatt BF. An endogenous sleep-inducing compound is a novel competitive inhibitor of fatty acid amide hydrolase. Bioorganic and medicinal Chemistry Letters 1998;8(6):613–8.
40. Khanolkar AD, Makriyannis A. Structure activity relationships of anandamide, an endogenous cannabinoid ligand. Life Sci 1999;65:607–16.
41. Yu M, Ives D, Ramesha CS. Synthesis of prostaglandin E_2 ethanolamide from anandamide by cyclooxygenase-2. Journal of Biological Chemistry 1997;272(34):21181–6.
42. Ueda N, Yamamoto K, Yamamoto S, Tokunaga T, Shirakawa E, Shinkai M, Ogawa M, Sato T, Kudo I, Inoue K, Takizawa H, Nagano T, Hirobe M, Matsuki N, Satio H. Lipooxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist. Biochimica et Biophysica Acta 1995;1254(2):127–34.
43. Hampson AJ, Hill WA, Zan-Philips M, Makriyannis A, Leung E, Eglen RM, Bornheim LM. Anandamide

hydroxylation by brain lipooxygenase: metabolite structures and potencies at the cannabinoid receptor. Biochimica et Biophysica Acta 1995;1259(2):173–9.
44. Edgemond WS, Hillard CJ, Falck JR, Kearn CS, Campbell WB. Human platelets and polymorphonuclear leukocytes synthesize oxygenated derivatives of arachidonylethanolamide (anandamide): their affinities for the cannabinoid receptors and pathways of inactivation. Molecular Pharmacology 1998;54(1): 180–8.
45. Devane WA, Dysarz III FA, Johnson MR, Melvin LS, Howlett A. Determination and characterization of a cannabinoid receptor in rat brain. Molecular Pharmacology 1998;34(5):605–13.
46. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannbinoid receptor and functional expression of the cloned cDNA. Nature 1990;346(6284):561–4.
47. Gerard CM, Mollereau C, Vassart G, Parmentier M. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. The Biochemical journal 1991;279(Pt1):129–34.
48. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral cannabinoid receptor. Nature 1993;365(6441):61–5.
49. Kaminski NE, Abood ME, Kessler KF, Martin BR, Schatz AR. Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. Molecular Pharmacology 1992;42(5):736–42.
50. Bouaboula M, Rinaldi M, Carayon P, Carillon C, Delpech B, Shire D, Le Fur G, Casellas P. Cannabinoid-receptor expression in human leukocytes. European journal of Biochemistry 1993;214(1):173–80.
51. Chang MC, Berkery D, Schuel R, Layhock SG, Zimmerman AM, Zimmerman S, Schuel H. Evidence for a cannabinoid receptor in sea urchin sperm and its role in blockade of the acrosome reaction. Molecular Reproduction and Development 1993;36(4):507–16.
52. Schuel H, Goldstein E, Mechoulam R, Zimmerman AM, Zimmerman S. Anandamide (arachidonylethanolamide), a brain cannabinoid receptor agonist, inhibits fertilising capacity in sea urchin sperms by inhibiting the acrosome reaction. Proceedings of the National Academy of Sciences of the United States of America, vol. 91(16). 1994. pp. 7678–82.
53. Das SK, Paria BC, Chakraborty I, Dey SK. Cannabinoid-receptor signalling in the mouse uterus. Proceedings of the National Academy of Sciences of the United States of America, vol. 92(10). 1995. pp. 4332–6.
54. Kenney SP, Kekuda R, Prasad PD, Leibach FH, Devoe LD, Ganapathy V. Cannabinoid receptors and their role in the regulation of serotonin transporter in the human placenta. American journal of Obstetrics and Gynecology 1999;181(2):491–7.
55. Liu J, Gao B, Mirshahi F, Sanyal AJ, Khanolkar AD, Makriyannis A, Kunos G. Functional CB_1 cannabinoid receptors in human endothelial cells. The Biochemical journal 2000;346(Pt3):835–40.
56. Sugiura T, Kodaka T, Nakane S, Kishimoto S, Kondo S, Waku K. Detection of a cannabimimetic molecule, 2-arachidonoylglycerol, and cannabinoid CB_1 receptor mRNA in human vascular cells: is 2-arachidonoylglycerol a possible vasomodulator. Biochemical and Biophysical Research Communications 1998;243(3): 838–43.
57. Straiker AJ, Maguire G, Mackie K, Lindsey J. Localization of cannabinoid CB_1 receptors in the human anterior eye and retina. Investigative Ophthalmology and Visual Science 1999;40(10):2442–8.
58. Porcella A, Maxia C, Gessa GL, Pani L. The human eye expresses high levels of CB_1 cannabinoid receptors mRNA and protein. European journal of Neuroscience 2000;12(3):1123–7.
59. Condie R, Herring A, Koh WS, Lee M, Kaminski NE. Cannabinoid inhibition of adenylate cyclase-mediated signal transduction and interleukin 2 (IL-2) expression in the murine T-cell line, EL4.IL-2. Journal of Biological Chemistry 1996;271(22):13175–83.
60. Jeon YJ, Yang KH, Pulaski JT, Kaminski NE. Attenuation of inducible nitric oxide synthase gene expression by delta 9-tetrahydrocannabinol is mediated through the inhibition of nuclear factor kappa B/Rel activation. Molecular Pharmacology 1996;50(2):334–41.
61. Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proceedings of the National Academy of Sciences of the United States of America, vol. 92(8). 1995. pp. 3376–80.

62. Howlett AC. Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. Molecular Pharmacology 1985;27(4):429–36.
63. Howlett AC, Qualy JM, Khachatrian LL. Involvement of G_i in the inhibition of adenylate cyclase by cannabimimetic drugs. Molecular Pharmacology 1986;29(3):307–13.
64. Bidaut-Russell M, Devane WA, Howlett AC. Cannabinoid receptors and modulation of cyclic AMP accumulation in the rat brain. Journal of Neurochemistry 1990;55(1):21–6.
65. Houston DB, Howlett AC. Solubiliziation of the cannabinoid receptor from rat brain and its functional interaction with guanine nucleotide-binding proteins. Molecular Pharmacology 1993;43(1):17–22.
66. Howlett AC. Pharmacology of cannabinoid receptors. Annual review of Pharmacology and Toxicology 1995; 35:607–34.
67. Mackie K, Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastomaglioma cells. Proceedings of the National Academy of Sciences of the United States of America, vol. 89(9). 1992. pp. 3825–9.
68. Caulfield MP, Brown DA. Cannabinoid receptor agonists inhibit calcium currents in NG 108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. British journal of Pharmacology 1992;106(2):231–2.
69. Mechoulam R, Fride E, Di Marzo V. Endocannabinoids. European journal of Pharmacology 1998;359(1): 1–18.
70. Hampson AJ, Bornheim LM, Scanziani M, Yost CS, Gray AT, Hansen BM, Leonoudakis DJ, Bickler PE. Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. Journal of Neurochemistry 1998;70(2):671–6.
71. Shimasue K, Urushidani T, Hagiwara M, Nagao T. Effects of anandamide and arachidonic acid on specific binding of (+) -PN 200-110 diltiazem and (−) -desmethoxyverapamil to L-type Ca^{2+} channel. European journal of Pharmacology 1996;296(3):347–50.
72. Jarrahian A, Hillard CJ. Arachidonylethanolamide (anandamide) binds with low affinity to dihydropyridine binding sites in brain membranes. Prostaglandins, Leukotrines, and Essential Fatty Acids 1997; 57(6):551–4.
73. Tyrey L. delta 9-Tetrahydrocannabinol: a potent inhibitor of episodic luteinizing hormone secretion. Journal of Pharmacology and Experimental Therapeutics 1980;213(2):306–8.
74. Field E, Tyrey L. Delayed sexual maturation in the female rat during exposure to delta 9-Tetrahydrocannabinol. Life Sciences 1984;35(17):1725–30.
75. Nir I, Ayalon D, Tsafriri A, Cordova T, Lindner HR. Suppression of the cyclic surge of luteinizing hormone secretion and of ovulation in the rat by Δ^1-terahydrocannabinol. Nature 1973;243:470–1.
76. Ayalon D, Nir I, Cordova T, Bauminger S, Puder M, Naor Z, Kashi R, Zor U, Harell A. Acute effect of delta 1-tetrahydrocannabinol on the hypothalamic-pituitary-ovarian axis in the rat. Neuroendocrinology 1977;23 (1):31–42.
77. Wenger T, Croix D, Tramu G. The effect of prepubertal administration of marihuana (delta 9-tetrahydrocannabinol) on the onset of puberty and the postpubertal reproductive functions in female rats. Biology of Reproduction 1988;39(3):540–5.
78. Zoller LC, Carr K. Effects of daily administration of tetrahydrocannabinol on rat preovulatory follicles. A quantitative electron-microscopic analysis. Acta Anatomica 1988;131(4):265–70.
79. Smith CG, Besch NF, Smith RG, Besch PK. Effect of tetrahydrocannabinol on the hypothalamic-pituitary axis in the ovariectomized rhesus monkey. Fertility and Sterility 1979;31(3):335–9.
80. Wenger T, Toth BE, Juaneda C, Leonardelli J, Tramu G. The effects of cannabinoids on the regulation of reproduction. Life Sciences 1999;65(6–7):695–701.
81. Gonzalez S, Manzanares J, Berrendero F, Wenger T, Corchero J, Bisogno T, Romero J, Fuentes JA, Di Marzo V, Ramos JA, Fernandez-Ruiz J. Identification of endocannabinoids and cannabinoid CB_1 receptor mRNA in the pituitary gland. Neuroendocrinology 1999;70(2):137–45.
82. Wenger T, Fernandez-Ruiz JJ, Ramos JA. Immunocytochemical demonstration of CB_1 cannabinoid receptors in the anterior lobe of the pituitary gland. Journal of Neuroendocrinology 1999;11(11):837–8.
83. Fernandez-Ruiz JJ, Munoz RM, Romero J, Villanua MA, Makriyannis A, Ramos JA. Time course of the effect of different cannabimimetics on prolactin and gonadotrophin secretion: evidence for the presence of

CB_1 receptors in hypothalamic structures and their involvement in the effects of cannabimimetics. Biochemical Pharmacology 1997;53(12):1919–27.
84. Gonzalez S, Bisogno T, Wenger T, Manzanares J, Milone A, Berrendero F, Di Marzo V, Ramos JA, Fernandez-Ruiz JJ. Sex steroid influence on cannabinoid CB_1 receptor mRNA and endocannabinoid levels in the anterior pituitary gland. Biochemical and Biophysical Research Communications 2000;270(1):260–6.
85. Wenger T, Fragkakis G, Giannikou P, Probonas K, Yiannikakis N. Effects of anandamide on gestation in pregnant rats. Life Sciences 1997;60(26):2361–71.
86. Wenger T, Fragkakis G, Giannikou P, Yiannikakis N. The effects of prenatally administered endogenous cannabinoids on rat offspring. Pharmacology, Biochemistry and behavior 1997;58(5):537–44.
87. Patra PB, Wadsworth RM. Quantitative evaluation of spermatogenesis in mice following chronic exposure to cannabinoids. Andrologia 1991;23(2):151–6.
88. Kolodny RC, Masters WH, Kolodner RM, Toro G. Depression of plasma testosterone levels after chronic intensive marihuana use. New England journal of Medicine 1974;290:872–4.
89. Rosenkrantz H, Grant RJ, Fleischman RW, Baker JR. Marihuana-induced embryotoxicity in the rabbit. Fundamental and applied Toxicology 1986;7(2):236–43.
90. Paria BC, Huet-Hudson YM, Dey SK. Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. Proceedings of the National Academy of Sciences of the United States of America, vol. 90(21). 1993. pp. 10159–62.
91. Paria BC, Deutsch DD, Dey SK. The uterus is a potential site for anandamide synthesis and hydrolysis: differential profiles of anandamide synthase and hydrolase activities in the mouse uterus during the periimplantation period. Mol Reproduction and Development 1996;45(2):183–92.
92. Paria BC, Zhao X, Wang J, Das SK, Dey SK. Fatty-acid amide hydrolase is expressed in the mouse uterus during the periimplantation period. Biology of Reproduction 1999;60(5):1151–7.
93. Maccarrone M, De Felchi M, Bari M, Klinger F, Siracusa G, Finazzi-Agro A. Down-regulation of anandamide hydrolase in mouse uterus by sex hormones. European journal of Biochemistry 2000;267(10):2991–7.
94. Paria BC, Das SK, Dey SK. The preimplantation mouse embryo is a target for cannabinoid ligand-receptor signalling. Proceedings of the National Academy of Sciences of the United States of America, vol. 92(21). 1995. pp. 9460–4.
95. Yang ZM, Paria BC, Dey SK. Activation of brain-type cannabinoid receptors interferes with preimplantation mouse embryo development. Biology of Reproduction 1996;55(4):756–61.
96. Paria BC, Ma W, Andrenyak DM, Schmid PC, Schmid HH, Moody DE, Deng H, Makriyannis A, Dey SK. Effects of cannabinoids on preimplantation mouse embryo development and implantation are mediated by brain-type cannabinoid receptors. Biology of Reproduction 1998;58(6):1490–5.
97. Paria BC, Dey SK. Ligand-receptor signalling with endocannabinoids in preimplantation embryo development and implantation. Chemistry and Physics of Lipids 2000;108(1–20):211–20.
98. Wang J, Paria BC, Dey SK, Armant DR. Stage-specific excitation of cannabinoid receptor exhibits differential effects on mouse embryonic development. Biology of Reproduction 1999;60(4):839–44.
99. Paria BC, Song H, Wang X, Schmid PC, Krebsbach RJ, Schmid HHO, Bonner TI, Zimmer A, Dey SK. Dyregulated cannabinoid signalling disrupts uterine receptivity for embryo implantation. Journal of Biological Chemistry 2001;276(23):20523–8.
100. Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agro A. Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage. The Lancet 2000;355(9212): 1326–9.
101. Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agro A. Progesterone up-regulates anandamide hydrolase in human lymphocytes: role of cytokines and implications for fertility. The journal of Immunology 2001;166:7183–9.
102. Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S. Defective production of both leukaemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. Nature Medicine 1998;4(9):1020–4.
103. Lockwood CJ. Prediction of pregnancy loss. The Lancet 2000;355(9212):1292–3.